

# Community-acquired culture-negative endocarditis: clinical characteristics and risk factors for mortality



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## SUMMARY

**Objectives:** We studied the clinical characteristics, in-hospital mortality, and long-term prognosis of patients with culture-negative endocarditis.

**Methods:** In total, 221 episodes of definite endocarditis were studied (2004–2009). We compared the clinical, laboratory, and echocardiography characteristics and the survival rates of patients with culture-negative and culture-positive endocarditis. Survival after hospital discharge was evaluated using the Kaplan–Meier method and coefficient of mortality comparisons.

**Results:** Culture-negative endocarditis occurred in 51/221 (23.1%) episodes. Compared with the culture-positive endocarditis patients, the time elapsed between admission and initiation of antibiotic therapy was longer in patients with culture-negative endocarditis ( $p < 0.001$ ), and these patients also had lower C-reactive protein levels at admission ( $p < 0.001$ ). In-hospital mortality rates were not different between culture-negative and culture-positive patients. After hospital discharge, there was also no significant difference between groups in survival curves ( $p = 0.471$ ). Severe sepsis (adjusted prevalence ratio 3.32,  $p = 0.010$ ) and diabetes mellitus (adjusted prevalence ratio 2.32,  $p = 0.009$ ) were independently associated with in-hospital death in culture-negative patients.

**Conclusions:** Culture-negative endocarditis patients presented with lower levels of C-reactive protein at admission and required more time for initiation of antibiotic therapy, although there was no difference in in-hospital mortality or long-term survival between culture-negative and culture-positive endocarditis patients. Diabetes mellitus and severe sepsis were associated with in-hospital death in patients with culture-negative endocarditis.

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## 1. Introduction

Culture-negative endocarditis remains a diagnostic and therapeutic challenge. Although new techniques, such as those involving molecular biology, are highly sensitive for the identification of causative microorganisms when applied to cardiac valve in surgically treated patients, their sensitivity is low when peripheral blood is analyzed.<sup>1–3</sup>

The clinical characteristics and prognosis associated with this condition may vary according to different epidemiological and clinical factors. Empirical antimicrobial treatment is based on epidemiological data and previous exposure to microorganisms,<sup>4–6</sup> although

unusual or fastidious microorganisms can also cause endocarditis, especially in cases of community-acquired endocarditis.<sup>7,8</sup>

We hypothesized that culture-negative endocarditis cases may present different clinical characteristics and prognoses compared to cases with an identified microbial pathogen. Thus, we compared the clinical characteristics, in-hospital mortality, and long-term prognoses between endocarditis patients with and without a causative microorganism identified in blood culture.

## 2. Methods

### 2.1. Study setting, clinical setting, and patients

Using an inception cohort, we studied 221 cases of infective endocarditis admitted to a 514-bed tertiary cardiology hospital

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between January 2004 and January 2009. Participants were included in this inception cohort consecutively at diagnosis of endocarditis, which was considered the time at which active parenteral antibiotic treatment was started. The inclusion criteria were age >18 years and 'definite' endocarditis (according to the modified Duke criteria<sup>9</sup>) involving a native or prosthetic valve. The exclusion criteria were as follows: (1) patients with prosthetic valve endocarditis in the first postoperative year, (2) hemodialysis patients, and (3) nosocomial endocarditis patients (i.e., those with symptoms attributed to endocarditis that started 72 h after hospital admission or within 6 months of a hospital-associated invasive procedure<sup>10,11</sup>).

Our hospital follows international recommendations for specific antibiotic therapy for endocarditis.<sup>12</sup> Empirical antibiotic treatment for culture-negative endocarditis is the association of intravenous (IV) penicillin G (24 million units/day in divided doses every 4 h) plus IV oxacillin (12 g/day in divided doses every 4 h)  $\pm$  IV gentamicin (1 mg/kg every 8 h). The patients ranged in age from 18 to 90 years (mean 53 years, standard deviation 18.4 years); 144 (65.2%) were male and 77 (34.8%) were female. Native valve endocarditis occurred in 114 patients and valvular heart disease occurred in 79 patients (chronic rheumatic heart disease occurred in 29, mitral valve prolapse in 19, and congenital heart disease in 22). Nine patients presented evidence of previous endocarditis. Prosthetic valve endocarditis occurred in 107 patients. Left-sided endocarditis occurred in 209 patients (mitral or aortic), and right-sided endocarditis occurred in 12 patients.

## 2.2. Diagnostic work-up

After the clinical examination, three sets of blood cultures (aerobic/anaerobic) containing 5–10 ml of blood in each sample were drawn by independent punctures at least 30 min apart using an aseptic technique. The blood samples were incubated for 5 days. Automated BACTEC (BD Diagnostics, Sparks, MA, USA) and VITEK 2 (bioMérieux, Marcy l'Etoile, France) systems were used for the identification of microorganisms and susceptibility testing. In the event that a microorganism was not identified in the blood culture, indirect immunofluorescence assays were performed for *Bartonella henselae*, *Bartonella quintana*, and *Coxiella burnetii*. Sera that showed fluorescence at a dilution  $\geq 1:800$  for *B. henselae*, *B. quintana*, or *C. burnetii* anti-phase I were considered positive.<sup>13,14</sup>

When available, surgically removed and formalin-fixed, paraffin-embedded 5- $\mu$ m thick valve tissue sections were submitted to *B. henselae*, *B. quintana*, and *C. burnetii* immunohistochemistry.

## 2.3. Variables studied

The following variables were studied: (1) demographic data (sex and age); (2) predisposing diseases (valvular heart disease, cyanotic congenital heart disease, prosthetic cardiac valve, and previous endocarditis); (3) comorbidities (diabetes mellitus, arterial hypertension, creatinine clearance <30 ml/min, AIDS, and patients undergoing chemotherapy); (4) antimicrobial administration prior to blood draw (these data were obtained prospectively by interview with culture-negative patients); (5) endocarditis characteristics at admission (type and duration of symptoms, hospitalization time until initiation of antibiotic therapy for endocarditis, location of endocarditis (right or left side), and native or prosthetic valve); (6) C-reactive protein level on admission, vegetation on echocardiography, left ventricular ejection fraction, and valve regurgitation; (7) endocarditis treatment (valve surgery and the use of aminoglycoside combined antibiotic treatment); (8) complications (severe sepsis at admission (according to the criteria of Bone et al.<sup>15</sup>), the occurrence of renal failure during hospital stay (Acute Kidney Injury Network

criteria<sup>16</sup>), atrioventricular block, myocardial abscess, cardiac fistula, glomerulonephritis, and embolization); (9) follow-up (all patients were contacted by telephone at the end of follow-up – February 1, 2013); (10) relapse (defined as a new episode of endocarditis within 6 months after the end of treatment, with a negative blood culture or culture with the same microorganism identified previously<sup>17</sup>); in these cases, only the first episode was included.

## 2.4. Statistical analysis

Qualitative variables were compared between study groups using the Chi-square test or Fisher's exact test. Univariate analysis was performed to establish the prevalence ratio between clinical and laboratory variables as well as in-hospital mortality, with the corresponding 95% confidence intervals (95% CI). All variables with  $p < 0.20$  in the univariate analysis were tested in the multivariate Cox regression model with robust variance for transversal analysis (stepwise forward). After hospital discharge, the probability of survival was evaluated using the Kaplan–Meier method, and survival was compared between the negative and positive culture groups using the log-rank test. These analyses were performed using Stata 11.0 (StataCorp LP, College Station, TX, USA).

## 2.5. Ethical aspects

This study was approved by the ethics committee on human research of the Heart Institute (InCor), University of Sao Paulo Medical School.

# 3. Results

## 3.1. Clinical characteristics

We identified the causative microorganisms in 170 episodes of endocarditis, whereas 51 (23.1%) blood cultures were negative. Of the culture-negative patients, 24 (47.1%) received antibiotic therapy prior to the blood draw, including five cases (20.8%) at our institution, 18 (75%) during a previous hospitalization, and one (4.2%) who used self-medication.

Comorbidities were diagnosed in 152 (68.3%) patients. These conditions included diabetes mellitus in 25 patients (11.3%), hypertension in 91 patients (41.2%), heart failure in 78 patients (35.3%), chronic renal failure in 15 patients (6.8%), and chronic obstructive pulmonary disease in four patients (1.8%).

The following microorganisms were identified: viridans type streptococci in 81 (47.6%) patients, *Enterococcus spp* in 20 patients (11.7%), *Streptococcus bovis* in 17 patients (10%), *Staphylococcus aureus* in 14 patients (8.2%), other streptococci in 11 patients (6.5%), HACEK microorganisms in 13 patients (7.7%), coagulase-negative staphylococci in six patients (3.5%), and *Candida spp*, *Bacteroides fragilis*, *Citrobacter diversus*, *Salmonella spp*, *Serratia marcescens*, *Flavobacterium spp*, and *Corynebacterium spp* in one patient each.

Patients with negative blood cultures presented *Bartonella spp* infection (10 patients) or *C. burnetii* infection (four patients). Immunohistochemical valvular pathology confirmed these findings in nine patients who were submitted to surgical treatment.

## 3.2. Culture-negative endocarditis vs. culture-positive endocarditis

Table 1 describes the clinical characteristics of patients with culture-negative and culture-positive endocarditis. Interestingly, using the median value to categorize continuous variables, the levels of C-reactive protein were lower (median 58 mg/l vs. median 106 mg/l,  $p < 0.001$ ) and the time elapsed between admission and

**Table 1**

Clinical and laboratory characteristics of community-acquired endocarditis according to the identification of causative microorganisms in culture

Factor	Total number	Microorganisms on culture		p-Value <sup>a</sup>
		Identified, n (%)	Not identified, n (%)	
Male gender	221	108 (63.5)	36 (70.5)	0.353
Age ≥60 years	221	74 (43.5)	16 (31.4)	0.121
Valvular heart disease	221	144 (84.7)	40 (78.4)	0.293
Prosthetic valve endocarditis	221	85 (50.0)	22 (43.1)	0.427
Comorbidities	221	118 (69.4)	34 (66.7)	0.711
Duration of symptoms ≥30 days	220	75 (44.4)	28 (54.9)	0.187
Fever	220	153 (90.5)	44 (86.3)	0.384
Glomerulonephritis	183	34 (25.0)	1 (21.3)	0.607
C-reactive protein ≥80 mg/l	151	78 (68.4)	14 (37.8)	0.001
Severe sepsis	221	61 (35.9)	19 (37.3)	0.858
Time between admission and initiation of antibiotic therapy for IE >2 days	221	27 (15.9)	16 (31.4)	0.014
Right side IE	219	9 (5.4)	3 (5.9)	>0.999 <sup>b</sup>
Left side IE	221	161 (94.7)	46 (90.2)	>0.999 <sup>b</sup>
Vegetation on echocardiography	219	129 (76.8)	42 (82.3)	0.400
Renal failure	221	85 (50.0)	29 (53.9)	0.390
Atrioventricular block	221	28 (16.5)	4 (7.8)	0.125
Myocardial abscess	221	44 (25.9)	15 (29.4)	0.617
Cardiac fistula	221	4 (2.4)	4 (7.8)	0.085 <sup>b</sup>
Embolization	221	58 (34.1)	18 (35.3)	0.877
Aminoglycoside-containing therapy	221	133 (78.3)	40 (78.8)	0.976
Surgical treatment	221	92 (54.1)	24 (47.1)	0.376
In-hospital death	221	33.4%	31.4%	0.774

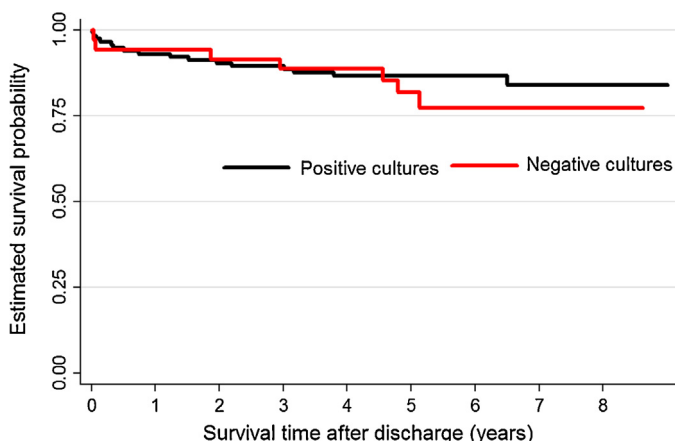
IE, infective endocarditis.

<sup>a</sup> Chi-square test.<sup>b</sup> Fisher's exact test.

initiation of antibiotic therapy was longer (median 2 days vs. median 0 days,  $p < 0.001$ ) in patients with culture-negative endocarditis compared with culture-positive endocarditis patients.

One hundred and sixteen patients (52.4%) were submitted to surgical treatment. The global in-hospital mortality rate was 33% (95% CI 26.9–39.7), and this rate was not different ( $p = 0.774$ ) between culture-negative endocarditis (31.4%, 95% CI 19.1–46.0) and culture-positive endocarditis patients (33.4%, 95% CI 26.5–41.2). Four patients experienced relapse, including two in the culture-negative group and two in the culture-positive group.

The mean follow-up time of patients after hospital discharge was 6 years (standard deviation  $\pm 1.6$  years). Five years after discharge, 85.3% of patients remained alive. No information could be obtained concerning the outcome in six patients (loss to follow-up 4.1%). The comparison of survival time after hospital discharge was also not significantly different ( $p = 0.471$ ) between the positive and negative culture groups, as demonstrated by the Kaplan–Meier curves (Figure 1).



**Figure 1.** Kaplan–Meier survival curve for endocarditis according to culture results ( $p = 0.471$ ).

### 3.3. In-hospital prognosis of patients with culture-negative endocarditis

Survival data for the negative blood culture patients are presented in Table 2. Multivariate Cox regression revealed that only severe sepsis (adjusted prevalence ratio 3.32, 95% CI 1.34–8.23;  $p = 0.010$ ) and diabetes mellitus (adjusted prevalence ratio 2.32, 95% CI 1.23–4.37;  $p = 0.009$ ) were associated with in-hospital death (Table 2).

## 4. Discussion

In our study, the frequency of culture-negative endocarditis was 22.5% (95% CI 17.2–28.5). Previous endocarditis case series have reported frequencies of negative cultures ranging from 12% to 60%.<sup>18–23</sup> Failure to culture the microorganism responsible for endocarditis may be the result of antimicrobial treatment prior to blood culture or infection with highly fastidious bacteria or with nonbacterial pathogens.<sup>8</sup> In this study, 47% of patients with culture-negative endocarditis were treated with antimicrobial drugs prior to blood draw, and the majority of these cases (75%) received the first antibiotic dose prior to hospital admission. As a referral hospital, most patients at our hospital had already received antibiotics at the time of admission.

We did not observe a lower frequency of fever in patients with negative blood cultures, as reported previously.<sup>24–26</sup> However, the lower values of C-reactive protein in the culture-negative endocarditis patients may be indicative of a less intense systemic inflammatory response secondary to fastidious infections. Our study found no differences between the groups (with positive and negative blood cultures) regarding cardiac or systemic complications of endocarditis. However, previous authors have observed a lower frequency of complications such as embolism<sup>27</sup> and perivalvular abscess<sup>24,25,28</sup> among patients with culture-negative endocarditis.

We hypothesized that delays in identifying the causative organism and consequently administering the appropriate therapy could result in poor clinical outcomes. However, we found no

**Table 2**

Distribution of clinical and laboratory characteristics of culture-negative endocarditis patients according to in-hospital mortality

Factor	Survivors (n = 35)	Non-survivors (n = 16)	Prevalence ratio	95% CI	p-Value
Male gender	25 (71.4%)	11 (68.7%)	0.92	0.38–2.20	0.846
Age ≥60 years	7 (20%)	9 (56.2%)	2.81	1.27–6.25	0.011
Valvular heart disease	26 (74.2%)	14 (87.5%)	1.93	0.51–7.32	0.337
Hypertension	12 (34.2%)	9 (42.9%)	1.84	0.81–4.18	0.148
Diabetes mellitus	1 (2.8%)	4 (80.0%)	3.07	1.58–5.94	0.001
Duration of symptoms ≥30 days	20 (57.1%)	8 (28.6%)	0.82	0.36–1.86	0.637
Fever	32 (91.4%)	12 (27.3%)	0.48	0.21–1.07	0.074
C-reactive protein ≥80 mg/l (n = 37)	10 (29.4%)	4 (28.6%)	0.82	0.30–2.26	0.704
Severe sepsis	8 (22.8%)	11 (57.9%)	3.71	1.51–9.12	0.004
Blood cultures collected without the prior use of antibiotics	19 (54.2%)	5 (20.8%)	0.51	0.21–1.27	0.149
Time between admission and diagnosis of IE >2 days	17 (48.5%)	10 (37.0%)	1.48	0.63–3.49	0.369
Right side IE	2 (3.6%)	1 (33.3%)	1.07	0.20–5.67	0.940
Left side IE	31 (60.7%)	15 (32.6%)	1.63	0.26–10.06	0.598
<i>Bartonella spp</i> infection	6 (17.1%)	4 (40.0%)	1.37	0.55–3.38	0.499
<i>Coxiella burnetii</i> infection	3 (8.5%)	1 (25.0%)	0.78	0.13–4.58	0.786
LVEF ≤45% (n = 50)	7 (20.5%)	5 (41.7%)	1.52	0.81 – 2.60	0.380
Moderate or severe valvular regurgitation	27 (77.1%)	11 (29.0%)	0.75	0.32–1.77	0.516
Aminoglycoside-containing therapy	28 (0.8%)	12 (30.0%)	0.83	0.33–2.08	0.683
Surgical treatment	20 (57.1%)	7 (25.9%)	0.69	0.30–1.58	0.383
Atrioventricular block	3 (8.5%)	1 (25.0%)	0.78	0.13–4.58	0.786
Myocardial abscess	9 (25.7%)	6 (40.0%)	1.44	0.63–3.27	0.384
Cardiac fistula	4 (11.4%)	2 (50.0%)	1.45	0.54–3.86	0.457
Embolization	9 (25.7%)	9 (50.0%)	2.36	1.05–5.31	0.038

CI, confidence interval; IE, infective endocarditis; LVEF, left ventricular ejection fraction.

significant difference in hospital mortality or long-term survival according to blood culture positivity, although the initiation of antibiotic therapy in the culture-negative endocarditis group required additional time. In the last 20 years, seven studies<sup>18,24–29</sup> have compared in-hospital mortality in patients with positive and negative endocarditis cultures, and only one study<sup>28</sup> found differences between these groups, with a lower mortality rate observed in culture-negative endocarditis patients. However, in that study,<sup>28</sup> 75% of cases with culture-negative endocarditis were classified as ‘possible’ and may therefore not represent true endocarditis cases, which could explain the improved survival observed for this group. We restricted our analysis to patients with community-acquired endocarditis that was classified as ‘definite’ according to the modified Duke criteria.<sup>9</sup>

Long-term survival was analyzed in a previous study<sup>29</sup> in patients with surgically treated endocarditis, and the results showed lower late survival rates in cases of culture-negative endocarditis.

We observed that antibiotic administration prior to blood culture collection did not affect hospital mortality among culture-negative endocarditis patients, as reported in other studies.<sup>18,26,30</sup> However, the presence of diabetes mellitus and the occurrence of severe sepsis on admission among culture-negative endocarditis patients were independently associated with in-hospital death. These two clinical conditions were described previously as factors associated with a poor prognosis in endocarditis patients, but few studies have examined these risk factors among culture-negative endocarditis patients.<sup>31–33</sup> In a univariate analysis, one previous study<sup>18</sup> found an association between hospital mortality and the following variables in patients with culture-negative endocarditis: definite endocarditis, the presence of heart failure, kidney failure, and embolism. In addition, Werner et al.<sup>28</sup> observed significantly higher hospital mortality rates in patients with culture-negative endocarditis who did not receive treatment with an aminoglycoside as part of empirical antibiotic therapy. In our series, 21% of culture-negative endocarditis patients did not receive an aminoglycoside as empirical treatment, and there was no difference in hospital mortality between culture-positive and culture-negative endocarditis patients regarding the use of aminoglycoside-containing therapy.

In conclusion, culture-negative endocarditis patients demonstrated similar clinical characteristics at admission, with the exception of lower C-reactive protein levels and a slightly longer time required for initiation of antibiotic therapy. In-hospital mortality and long-term survival were also similar between culture-negative and culture-positive endocarditis patients. Diabetes mellitus and severe sepsis were associated with in-hospital death in patients with culture-negative endocarditis.

**Conflict of interest:** All authors declare no financial or personal relationships with other people or organizations that could inappropriately influence (bias) their work.

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